Constitutively Active Receptors

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP I					
MSHR_mouse	melanocyte-stimulating hormone MSH	TMII	92 VSIVLETTIIL K	adenylyl cyclase activity/ HEK293, *stably transfected*	(Robbins, Nadeau et al. 1993)
CLASS A GROUP II					
5H1B_human	5-hydroxytryptamine$_{1B}$	C-terminus of IC3	313 RERKATKTLGI K,R,Q	binding of [^{35}S]GTP[S] / CHO-KI	(Pauwels, Gouble 1999)
5H2A_human	5-hydroxytryptamine$_{2A}$	C-terminus of IC3	322 NEQKACKVLGI K	IP production / COS-7	(Egan, Herrick-Davis et al. 1998)
2H2C_rat	5-hydroxytryptamine$_{2C}$	C-terminus of IC3	312 NEDDASKVLGI L	PI hydrolysis / COS-7	(Herrick-Davis, Egan et al. 1997)

CLASS A GROUP II					
A1AB_human	α_{1B}-adrenergic alpha 1B-AR	TMDI junction between TMDIII and IC2	63 FAIVGNILVIL A 142 CAISIDRYIGV A	IP / COS-7	(Scheer, Fanelli et al. 1997)
A1AB_human	α_{1B}-adrenergic alpha 1B-AR	junction between TMDIII and IC2	143 CAISIDRYIGV K	IP / COS-7	(Scheer, Costa et al. 2000)
A1AB_human	α_{1B}-adrenergic	TMIII carboxyl end of IC3 TMV	128 AVDVLCCTASI F 293 REKKAAKTLGI E 204 EEPFYALFSSLG V	IP / COS-1 IP arachidonic acid release IP / COS-1	(Perez, Hwa et al. 1996) (Hwa, Gaivin et al. 1997)
A1AB_human	α_{1B}-adrenergic	C-terminal IC3	293 SREKKAAKT X=19 different substitutions	PI / COS-7	(Kjelsberg, Cotecchia et al. 1992)
A1AB_human	α_{1B}-adrenergic	C-terminus IC3	288 293 KFSREKKAAKTLGI K H L	PI hydrolysis / rat fibroblast	(Allen, Lefkowitz et al. 1991)
A2AA_human	α_2C10-adrenergic alpha-2AAR	C-terminal IC3 loop	373(348?) EKRFTFVLAV X=F,A,C,E,K	adenylyl cyclase inhibition / HEK293	(Ren, Kurose et al. 1993)
ACM1_human	muscarinic Hm1 muscarinic acetylcholine M1	C-terminal IC3 loop junction	360 SLVKEKKAARTLS A	PI / HEK(U293)	(Högger, Shockley et al. 1995)
ACM2-human	muscarinic acetylcholine M2	junction of IC3 and TMVI	390 KKVTRTIL↑A 1-4 A inserted	IP production, inhibition of cAMP production / COS-7	(Liu, Blin et al. 1996)

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CLASS A GROUP II					
ACM3_rat	m3 muscarinic (rat) muscarinic acetylcholine M3	TMVI	507 TWTPYNIMVLVNT S	IP / COS-7	(Blüml, Mutschler et al. 1994)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	N-terminus to TMII TMVI	chimera composed of m2 1-69 m5 77-445 m2 391-466	β-gal / NIH 3T3	(Burstein, Spalding et al. 1996)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	TMVI	451 459 465 AILLA FIITW TPYNI MVLVST M L H C V S F T	β-gal; radioligand binding / NIH-3T3	(Spalding, Burstein et al. 1998)
ACM5_human	m5 muscarinic muscarinic acetylcholine M5	junction of TMVI and EC3	465 YNIMVLVSTFCDKCV X=V,F,R,K,+more	β-gal; radioligand binding / NIH-3T3	(Spalding, Burstein et al. 1997)
B1AR_human	β_1-adrenergic	C-terminus	389 RKAFQGLLCCA R	adenylyl cyclase; agonist binding / CHW	(Mason, Moore et al. 1999)
B2AR_human	β_2-adrenergic beta-2AR	C-terminal IC3 loop	266 272 FCLKEHKALKTLGI SR K A	adenylyl cyclase activation; agonist binding affinity / COS-7 or CHO	(Samama, Cotecchia et al. 1993); (Lefkowitz, Cotecchia et al. 1993)
DADR_human	dopamine D1A	carboxyl terminal IC3	264 SFKMSFKRETKVLKT I K 288 *from D1B receptor* APDTSIKKETKVLKT	adenylyl cyclase; cAMP accumulation / HEK293	(Charpentier, Jarvie et al. 1996)
DADR_human	dopamine D1	TMVI	286 FVCCWLPFFIL A	CAMP accumulation / COS-7	(Cho, Taylor et al. 1996)
HH2R_rat	histamine H_2	IC2	115 FMISLDRYCAV N,A	cAMP production / HEK-293	(Alewijnse, Timmerman et al. 2000)

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File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP III					
OPSD_human	opsin rhodopsin	TMII TMIII TMVII	90 FMVLGGFTSTLY D 113 GCNLEGFFAT Q 292 296 MTIPAFFAKSAAIY E G,E,M ^{292}Ala neutral a.a converted to carboxylate and competes with ^{113}Glu for salt bridge with ^{296}Lys	transducin; phosphorylation by rhodopsin kinase / COS	(Rim and Oprian 1995)
OPSD_human	opsin rhodopsin	TMIII	134 VVLAIERYVVV I,Q,S	transducin; radioligand binding / COS	(Acharya and Karnik 1996)
OPSD_human	opsin rhodopsin	TM6 *plus* TM3	257 RMVIIMVIAFL Y,N *plus* G113Q	transducin, GTPγS uptake / COS	(Han, Smith et al. 1998)
OPSD_human	opsin rhodopsin	TMVII IC2	296 PAFFAKSAAIY G X=E,M natural mutants + 10 different a.a. substitutions disrupts critical salt bridge between ^{296}Lys(TMVII) and ^{113}Glu(TMIII) 134 VVLAIERYVVV Q	transducin; radioligand binding / COS	(Govardhan and Oprian 1994); (Cohen, Yang et al. 1993) (Cohen, Yang et al. 1993)

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TRFR_mouse	thyrotropin-releasing hormone TRH-R	carboxyl tail	335 FRKLCNCKQK STOP	$^{45}Ca^{2+}$ efflux, $[Ca^{2+}]$ / Xenopus oocytes; IP formation / AtT20, *stably transfected*	(Matus-Leibovitch, Nussenzveig et al. 1995)

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File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP IV					
BRB2_human	bradykinin B_2 B2 bradykinin BK-2	TMIII TMVI	113 AIISMDLYSSI A 256 LLFIICNLPFQI F	IP production / COS-7	(Marie, Koch et al. 1999)

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File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS A GROUP V					
AG2R_rat	AT_{1A} Type-1A angiotensis II	TMIII	111 ASVSFNLYASV A disrupts ^{111}Asn(TMIII) - ^{292}Tyr(TMVII) interaction	phospholipase C; IP production / COS-7	(Groblewski, Maigret et al. 1997)
AG2R_rat	AT_{1A} Type-1A angiotensis II	C-terminus of TM7 other multiple mutations	305 LFYGFLGKKFK Q	IP production / HEK-293; intrcellular Ca^{2+} mobilization / CHO	(Parnot, Bardin et al. 2000)
FMLR_human	formylmethionylleucylphenylalanine (fMLPR)	IC1	51 LVIWVAGFRMTHTVTTISYLNKAVA LVVWVTAFEAKRTINAIWFLNLAVA (K above conflicts with SWISS-PROT database)	PI production; phospholipase C stimulation / COS-7	(Amatruda, Draga Graonic et al. 1995)
IL8B_human	interleukin-8 receptor B CXCR-2 chemokine	IC2	138 ACISVDRYLAIVH V	IP production; Ca^{2+} moblization and actin polymerization / NIH 3T3	(Burger, Burger et al. 1999)
LSHR_human	luteinizing hormone (LH)	IC3	564 MATNKDTKIAKK G	cAMP production / HEK293	(Kudo, Osuga et al. 1996)
LSHR_human	luteinizing hormone (LH)	TMVI	578 ILIFTDFTCMA G	cAMP production / COS-7	(Shenker, Laue et al. 1993)
LSHR_human	luteinizing hormone (LH)	TM6	571 577 KIAKKMAILIFTDFTCM I I	cAMP production / COS-7	(Kosugi, Van Dop et al. 1995)
LSHR_rat	luteinizing hormone / human chorionic gonadotropin (LH/hCG)	TMVI	556 ILIFTDFTCMA G, Y	cAMP production / HEK 293T	(Bradbury, Kawate et al. 1997; Bradbury and Menon 1999)
OPRD_mouse	delta opiod receptor	TM3	128 KVLSIDYYNMF A,K,H	adenylyl cyclase inhibition / COS-7	(Cavalli, Babey et al. 1999)
OXYR_human	oxytocin	IC2	137 LMSLDRCLAIC A	IP production / COS-7	(Fanelli, Barbier et al. 1999)

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PAFR_human	platelet-activating factor (PAF)	C-terminus of IC3	231 EVKRRALWMVCTVLAV R	IP production / COS-7	(Parent, Le G uill et al. 1996)
PAFR_human	platelet-activating factor (PAF)	TMIII	100 CLFFINTYCSV A	arachnidonate release, IP production, adenylyl cylcase inhibition / CHO	(Ishii, Izumi et al. 1997)
PE23_human	prostaglandin E_3, EP3III EP3IV	C-terminal tail	360 FCQEEFWGN FCQMRKRRLREQEEFWGN ↑truncated	inhibition of adenylyl cyclase / CHO-K1	(Jin, Mao et al. 1997)
PE23_mouse	prostaglandin E_3 EP3	carboxyl-terminal tail	336 KILLRKFCQIRDHT (3α) MMNHL (3β) ↑truncated	inhibition of adenylate cyclase / CHO, *stably expressed*	(Hasegawa, Negishi et al. 1996)
THRR_human	thrombin	EC2 loop	259 268 CHDVLNETLLEGYYAYY DLKD KDF I	$^{45}Ca^{2+}$ efflux, PI hydrolysis, reporter gene induction / COS-7	(Nanevicz, Wang et al. 1996)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	EC1 EC2	486 YYNHAIDWQTG F,M 568 YAKVSICLPMD T	inositol phosphate--diacylglycerol cascade / COS-7	(Parma, Van Sande et al. 1995)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMIII TMVII	509 ASELSVYTLTV A 672 YPLNSCANPFL Y	adenylyl cyclase activation / COS-7	(Duprez, Parma et al. 1994)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMV	597 VAFVIVCCCHV L	cAMP formation / COS-7 cells	(Esapa, Duprez et al. 1999)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	TMVII	677 CANPFLYAIFT V	cAMP formation / CHO cells	(Russo, Wong et al. 1999)
TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	IC3	613 621 VRNPQYNPGDKDTKIAK deletion	cAMP formation / COS-7	(Wonerow, Schoneberg et al. 1998)

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TSHR_human	thyrotropin (TSHR) thyroid stimulating hormone	IC3 / TMVI	623 632 KDTKIAKRMAVLIFTDFICM V I	cAMP activation / COS-7	(Paschke, Tonacchera et al. 1994)
V2R_human	vasopressin V2	IC2	136 LAMTLDRHRAI A	cAMP formation / COS-7	(Morin, Cotte et al. 1998)

File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS B GROUP I					
CALR_human	human calcitonin hCTR-1 hCTR-2	wild type (native) protein		adenylyl cyclase cAMP production / COS-1	(Cohen, Thaw et al. 1997)
CLASS B GROUP II					
PTRR_human	parathyroid hormone PTH / PTH-related peptide	junction of IC1 and TMII junction of IC3 and TMVI	223 TRNYIHMHLFL R,K 410 KLLKSTLVLMP C,others	cAMP accumulation / COS-7	(Schipani, Jensen et al. 1997)
CLASS B GROUP III					
GIPR_human	glucose-dependent insulinotropic peptide (GIP-R)	TMVI	340 VFAPVTEEQAR P	cAMP production / L293	(Tseng and Lin 1997)
GLR_rat	glucagon	junction of IC loop1 and TMII IC end of TMVI	178 TRNYIHGNLFA R 352 RLARSTLTLIP A	cAMP accumulation / COS-7	(Hjorth, Orskov et al. 1998)
VIPR_human	vasoactive intestinal peptide 1 (VIP)	junction of IC loop 1 and TMII junction of IC loop 3 and TMVI	178 RNYIHMHLFI R *requires functional integrity of the N-terminal EC domain* 343 LARSTLLLIP X= K,P	cAMP production / COS-7 or CHO	(Gaudin, Maoret et al. 1998) (Gaudin, Rouyer-Fessard et al. 1998)

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File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS C					
CASR_human	calcium-sensing	N-terminal EC	TLSFVAQNKIDSLNLDEFCNCSEHI *various substitutions, in multiple combinations*	IP / tsA	(Jensen, Spalding et al. 2000)

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File Name	Receptor	Mutation Site	Sequence	Assay / Cells	Reference
CLASS D					
Q74283 RCB2 C. cinereus	pheromone	TM6	229 PLSAYQIYLGT P	heterologous yeast assay	(Olesnicky, Brown et al. 1999)
STE2_yeast	pheromone α-factor	TM6	258 QSLLVPSIIFI LL	*lacZ* reporter gene	(Konopka, Margarit et al. 1996)
STE2_yeast	pheromone α-factor	double mutations TM5 and TM6	223 MSFVLVVKHILAIR C C 247 251 DSFHILLIMSCQSLL CC CC *double mutations* *double mutations*	*lacZ* reporter gene / yeast	(Dube, DeCostanzo et al. 2000)
STE3_yeast	pheromone a-factor	IC3	194 DVRDILHCTNS Q	β-galactosidase	(Boone, Davis et al. 1993)
STE2_yeast	pheromone α-factor	TM6	253 258 LIMSCQSLLVPSIIFI L LP	β-galactosidase	(Sommers, Martin et al. 2000)

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A Point Mutation Enhances MC-4 Receptor Constitutive Activity

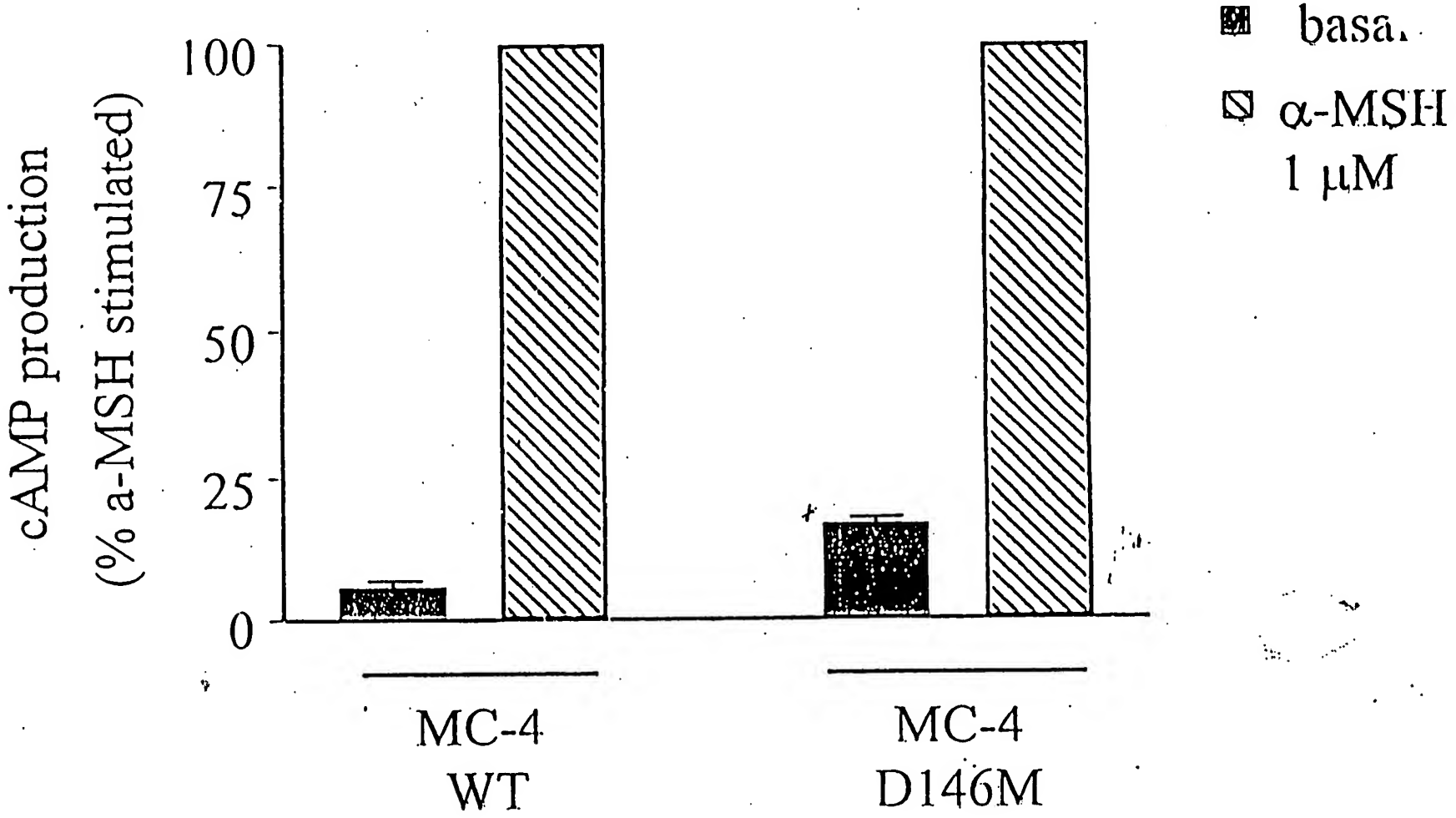


Figure 2

Light Emission Induced by the WT CCK-BR vs. a Constitutively Active Mutant

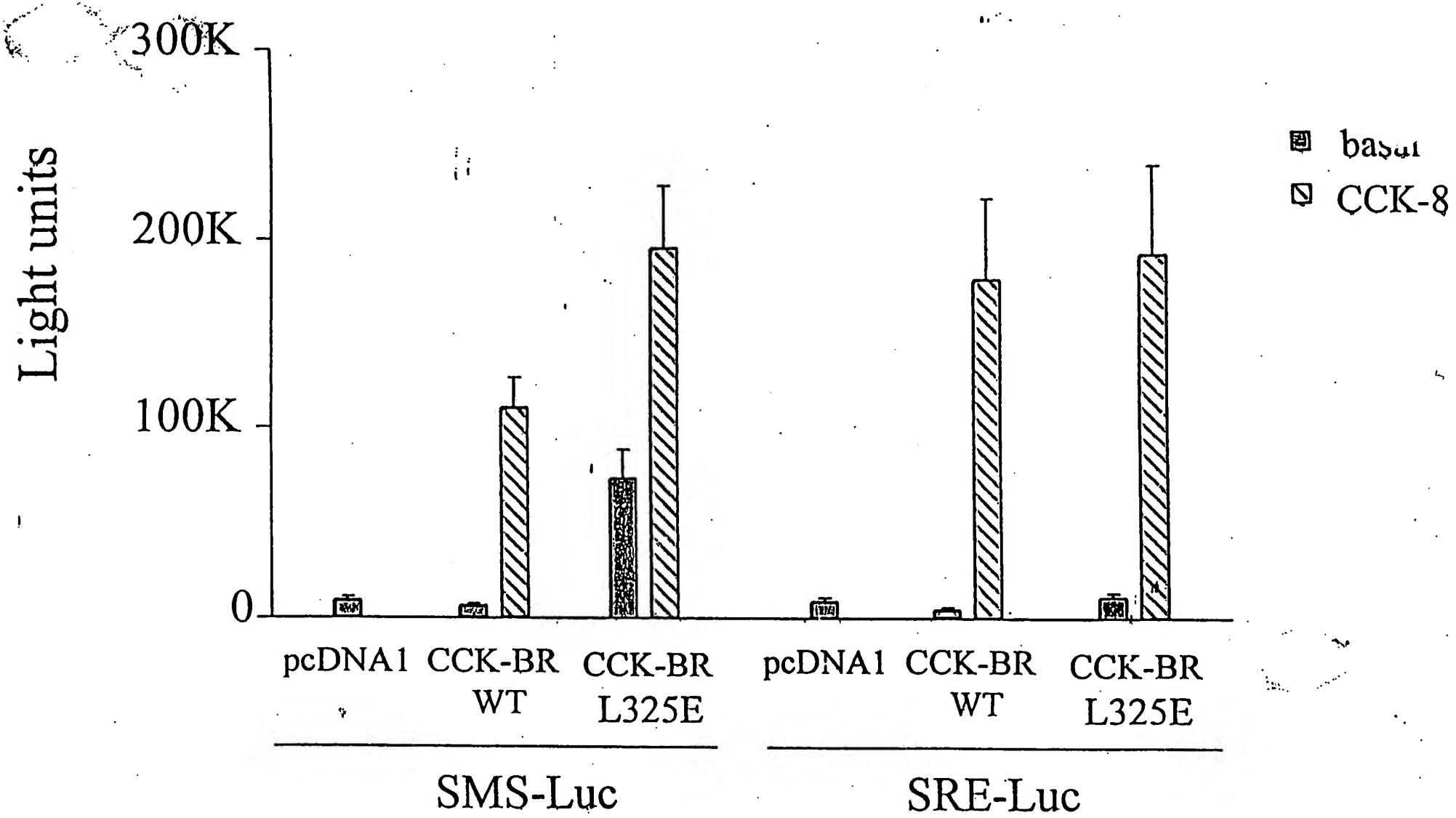


Figure 3

A Point Mutation Confers Constitutive Activity to the Rat μ Opiod Receptor

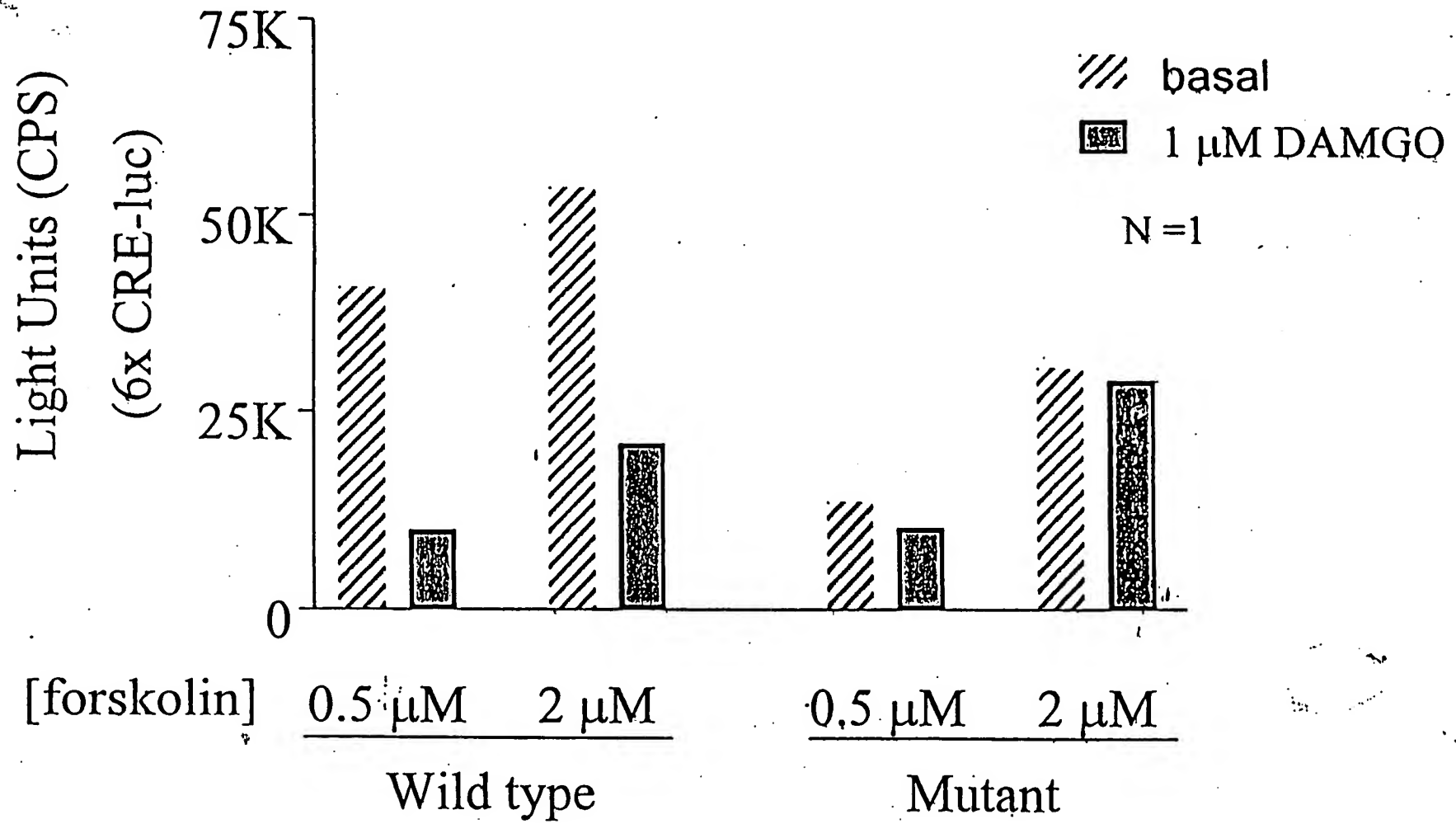


Figure 4

Forskolin Stimulated HEK293 Cells Transfected With pcDNA1 and a CRE-luc Construct

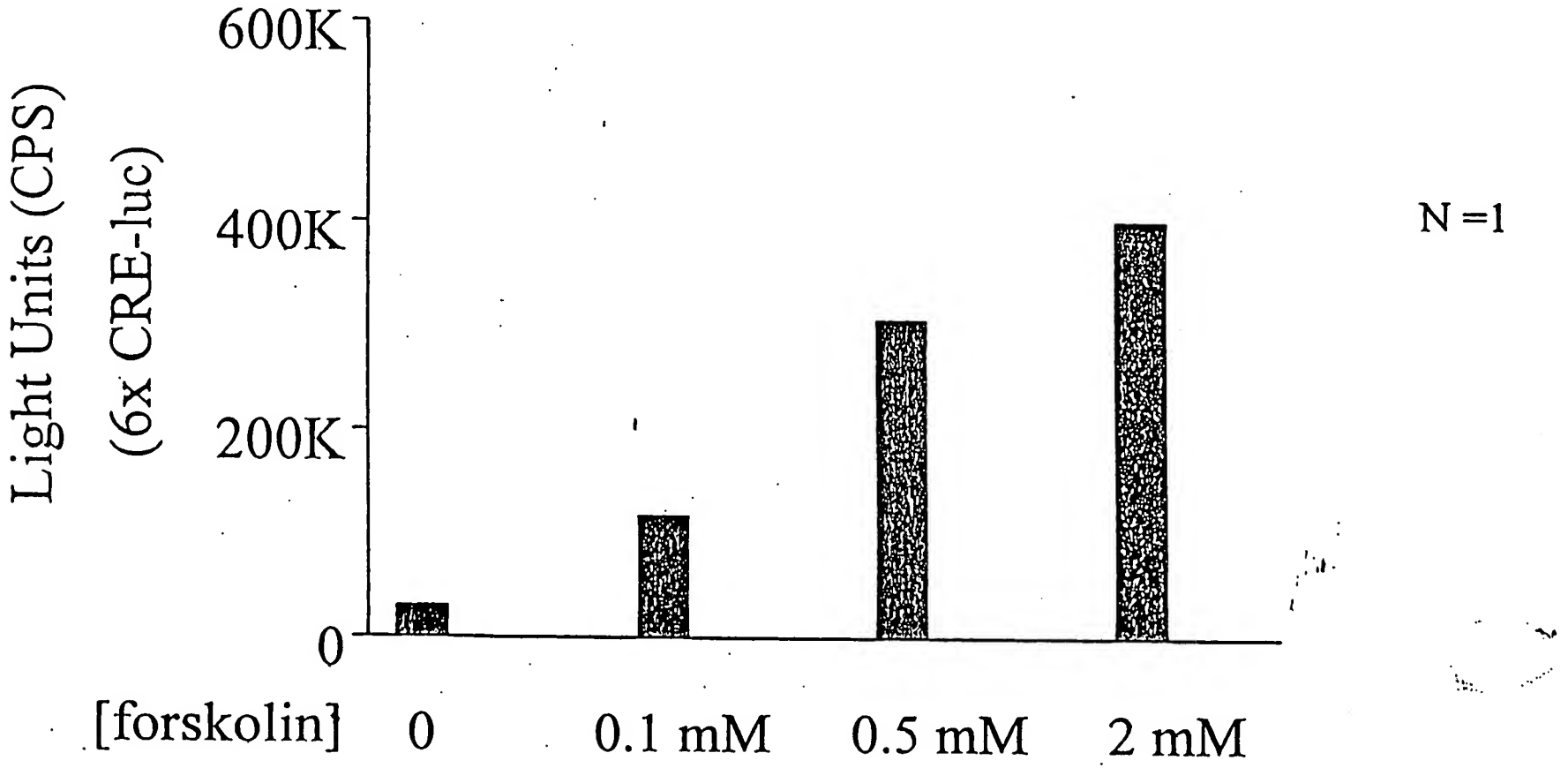


Figure 5

The Rat μ Opioid Receptor Signals Through Gαi

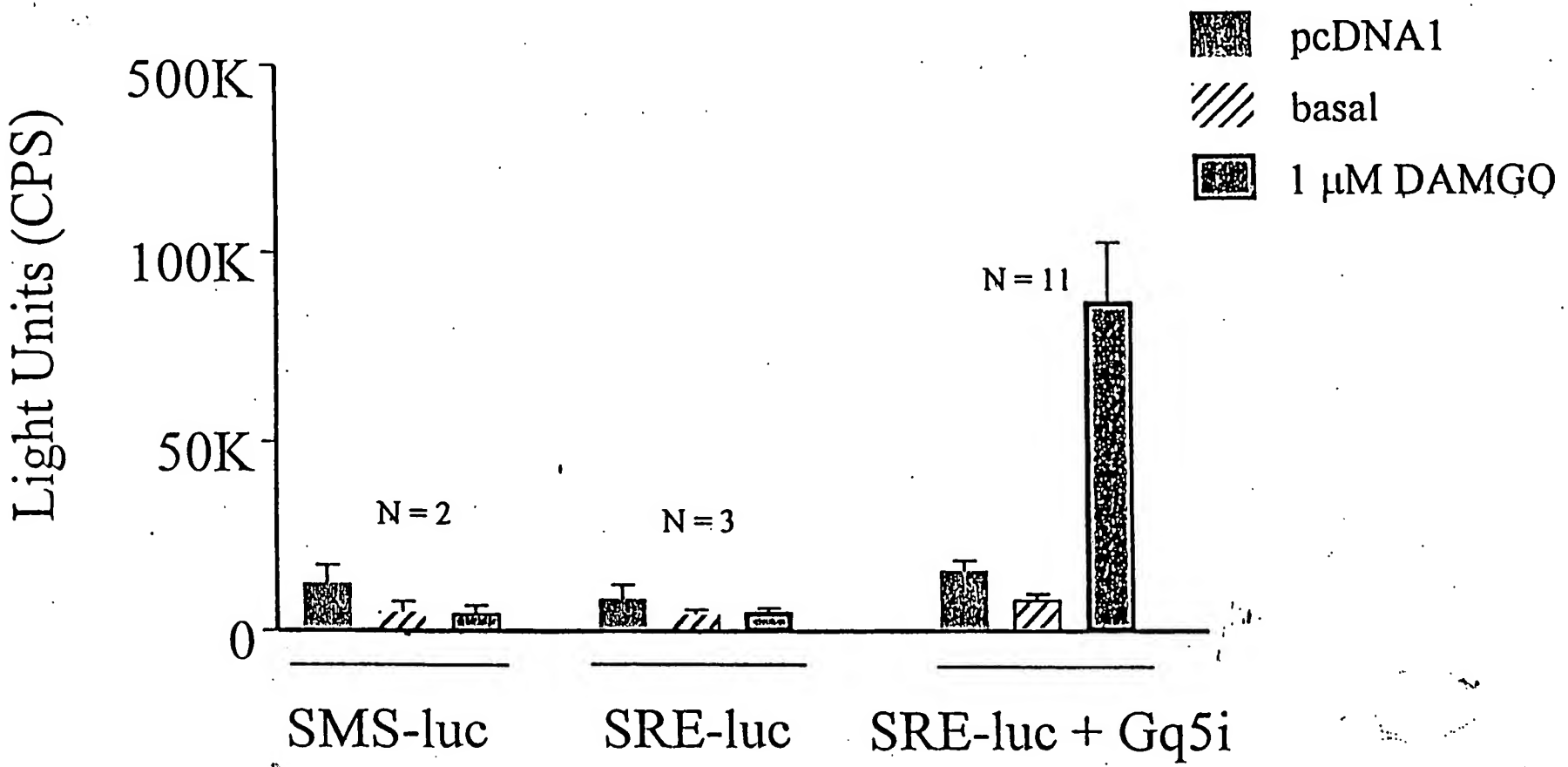


Figure 6

A Point Mutation Confers Constitutive Activity to the Rat μ Opioid Receptor

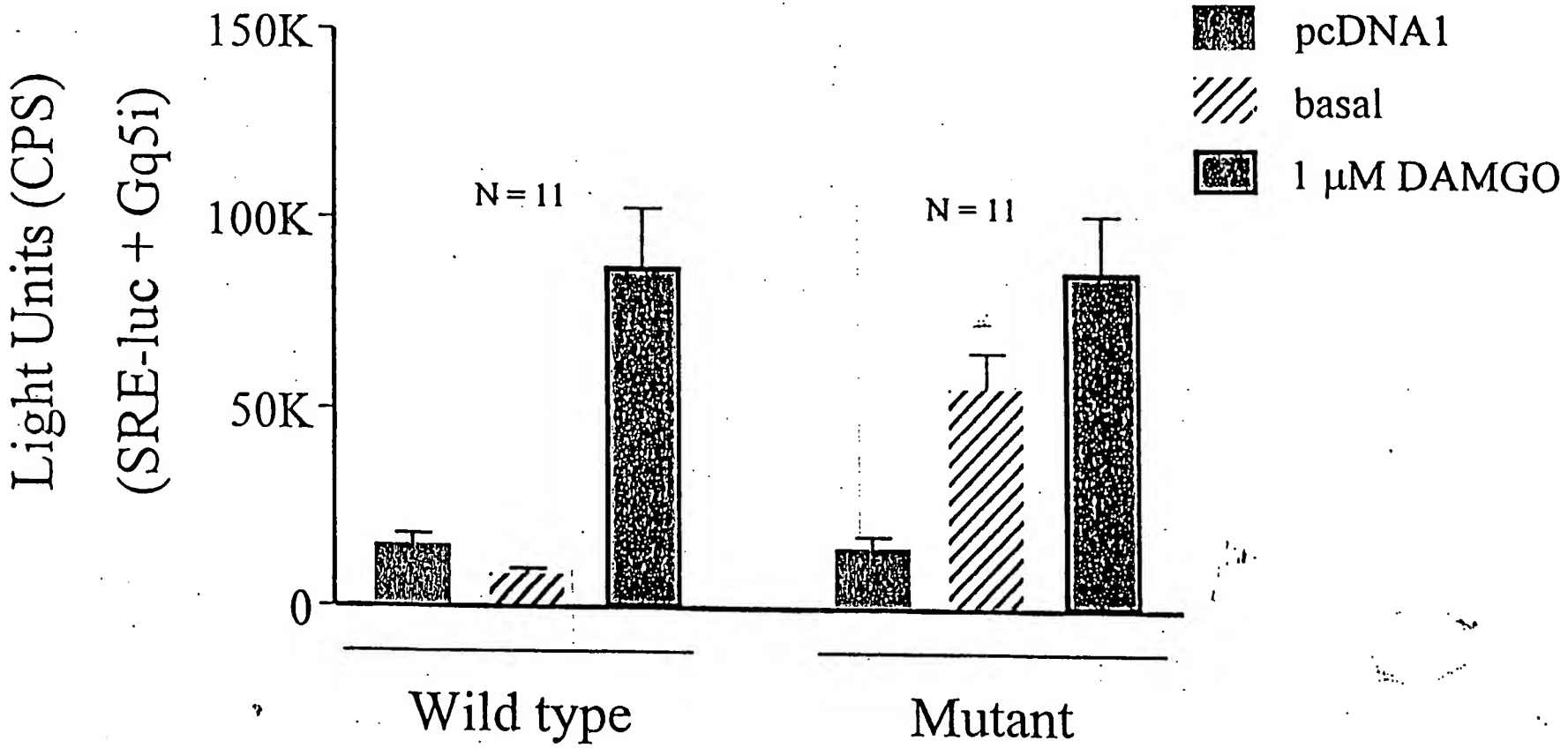


Figure 7

Target Residues Within Class I GPCRs

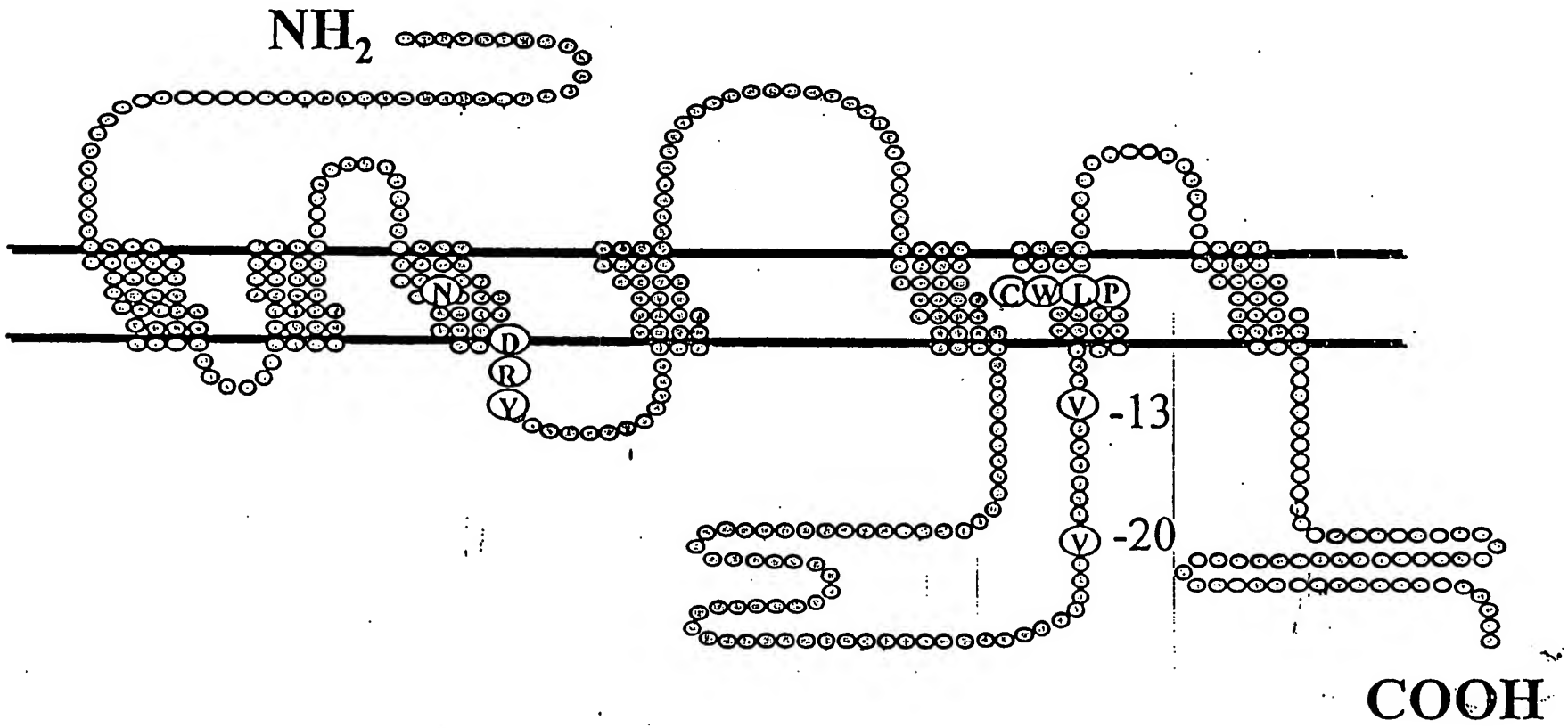
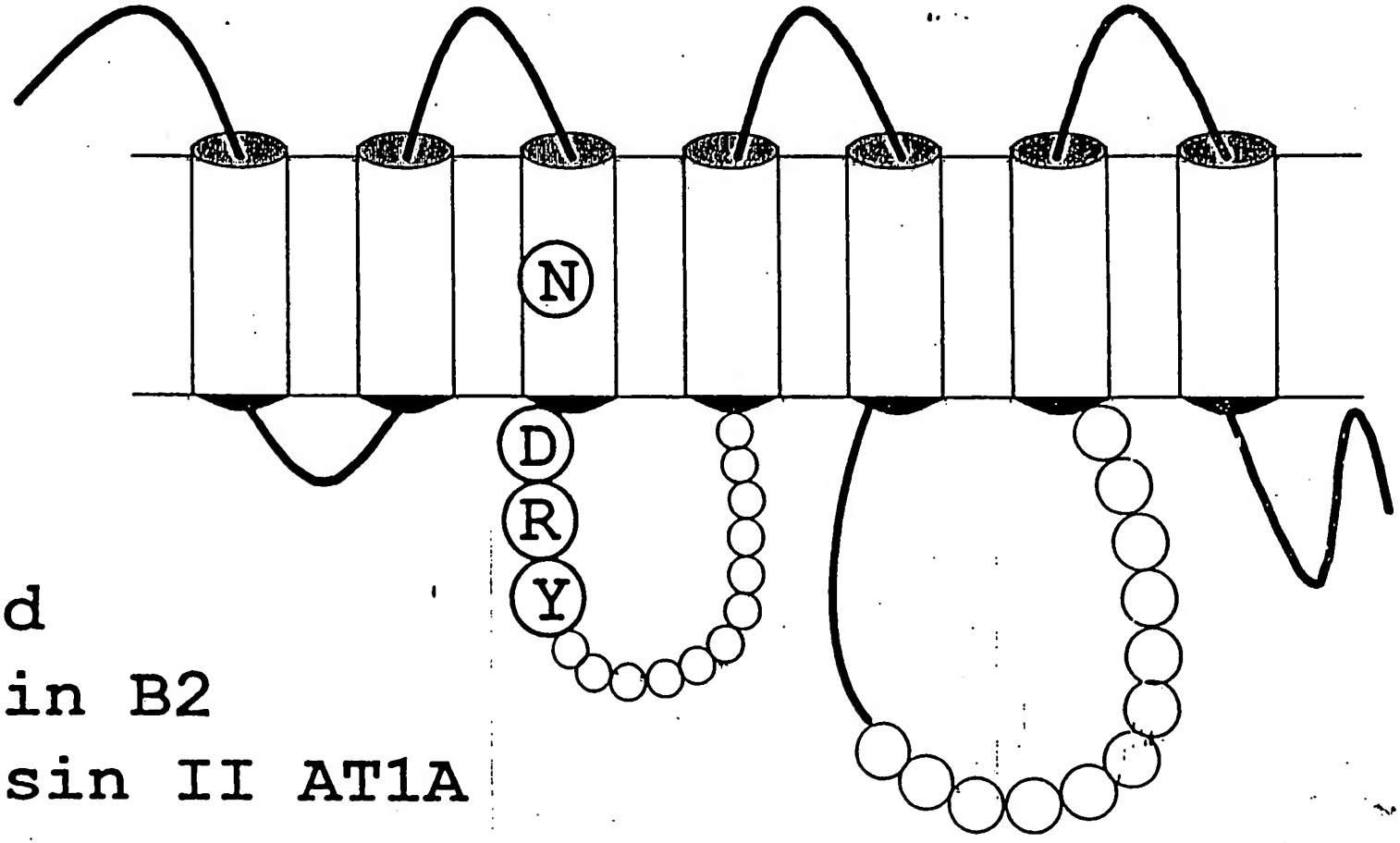


Figure 8

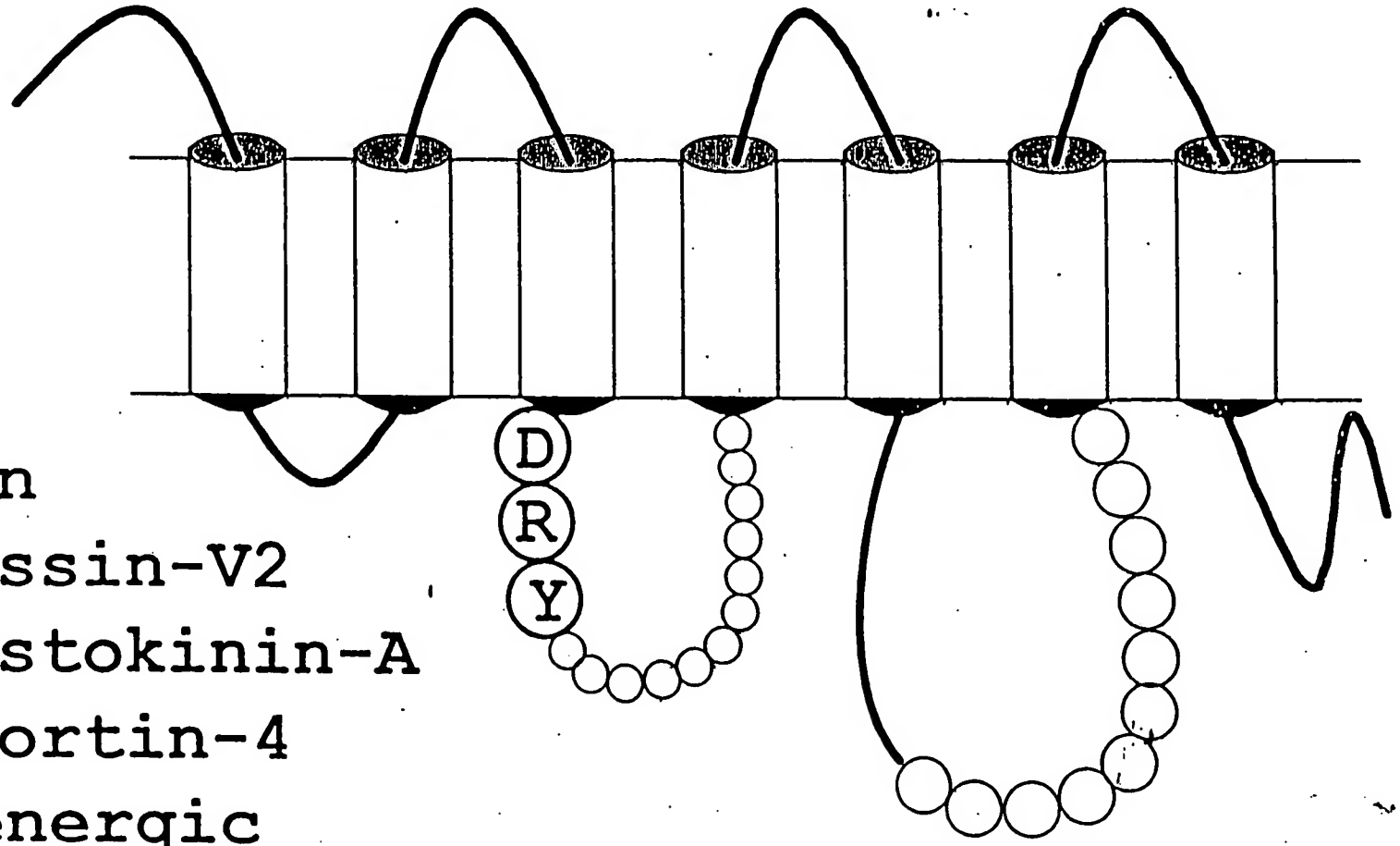
TMD III Asn (-14 from DRY) is a Target for Mutation Induced Constitutive Activity



mu opioid
bradykinin B2
angiotensin II AT1A

Figure 9

The 'DRY' Motif is a Target for Mutation Induced Constitutive Activity



oxytosin
vasopressin-V2
cholecystokinin-A
melanocortin-4
α_{1B} adrenergic

Figure 10

A Point Mutation Enhances MC-4 Receptor Constitutive Activity

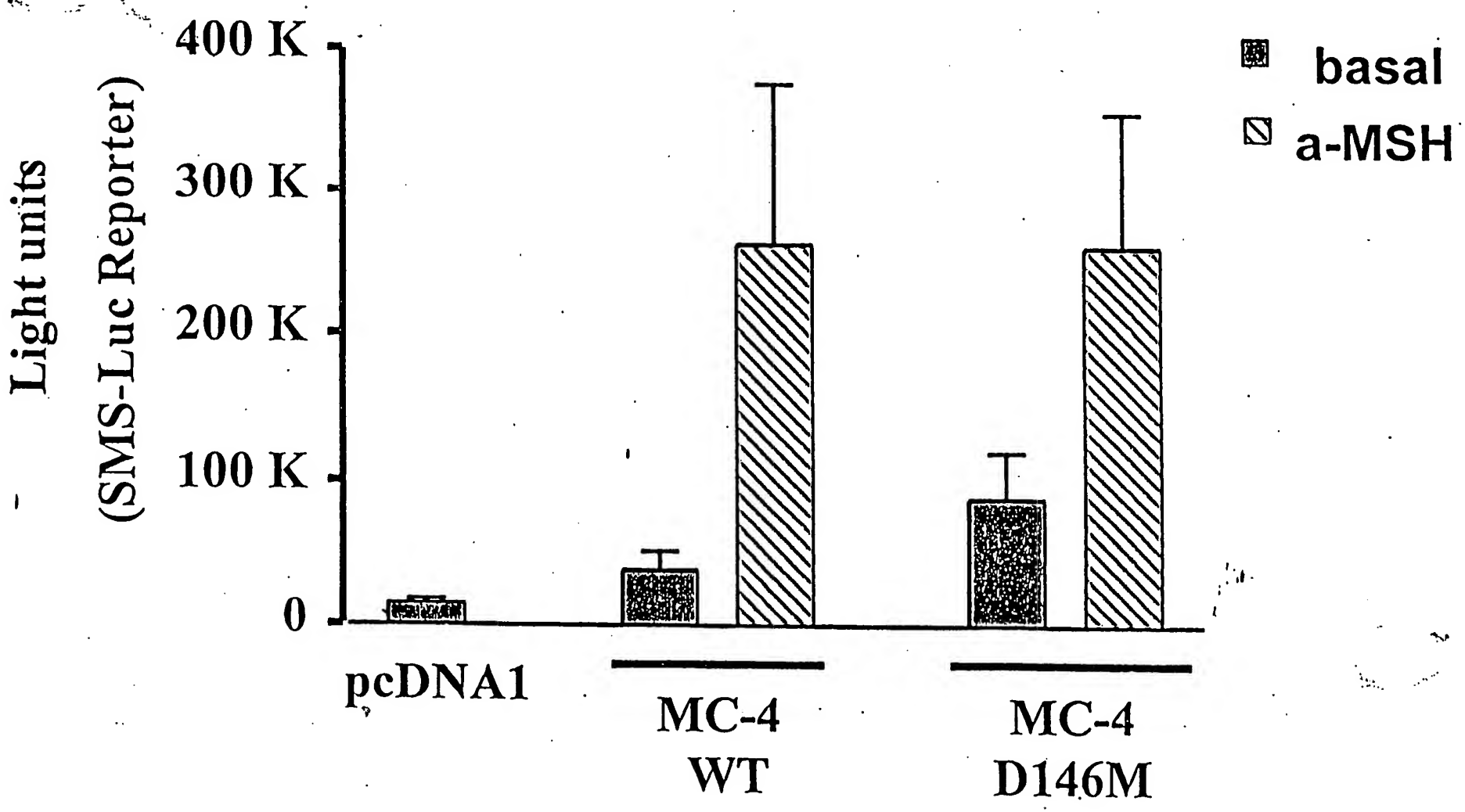
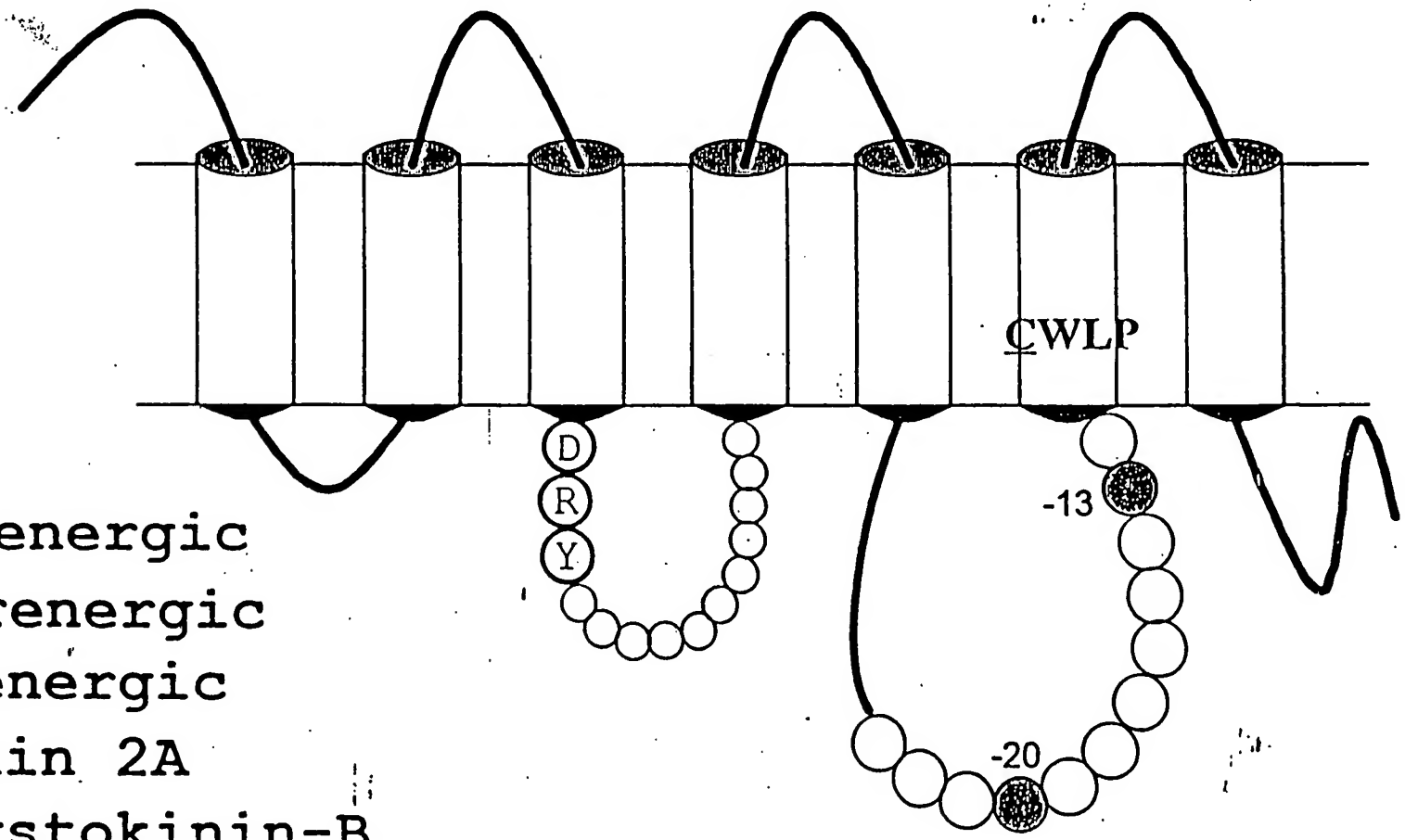


Figure 11

The -13 Position is a Target for Mutation Induced Constitutive Activity



$_{1A}$ adrenergic

$_{2C}$ adrenergic

$_{2}$ adrenergic

erotonin 2A

holecystokinin-B

latelet activating factor

hyroid stimulating hormone

Figure 12

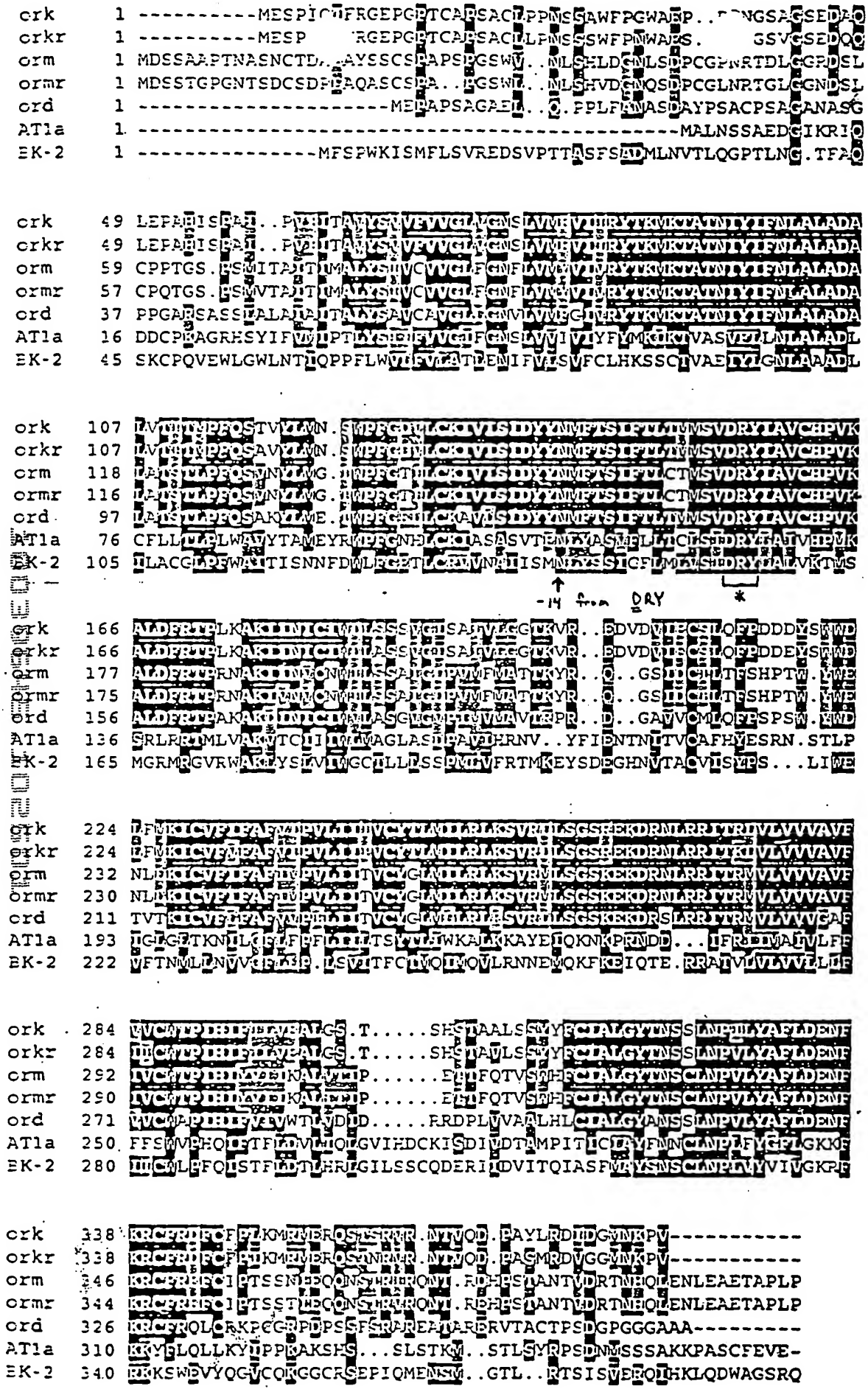
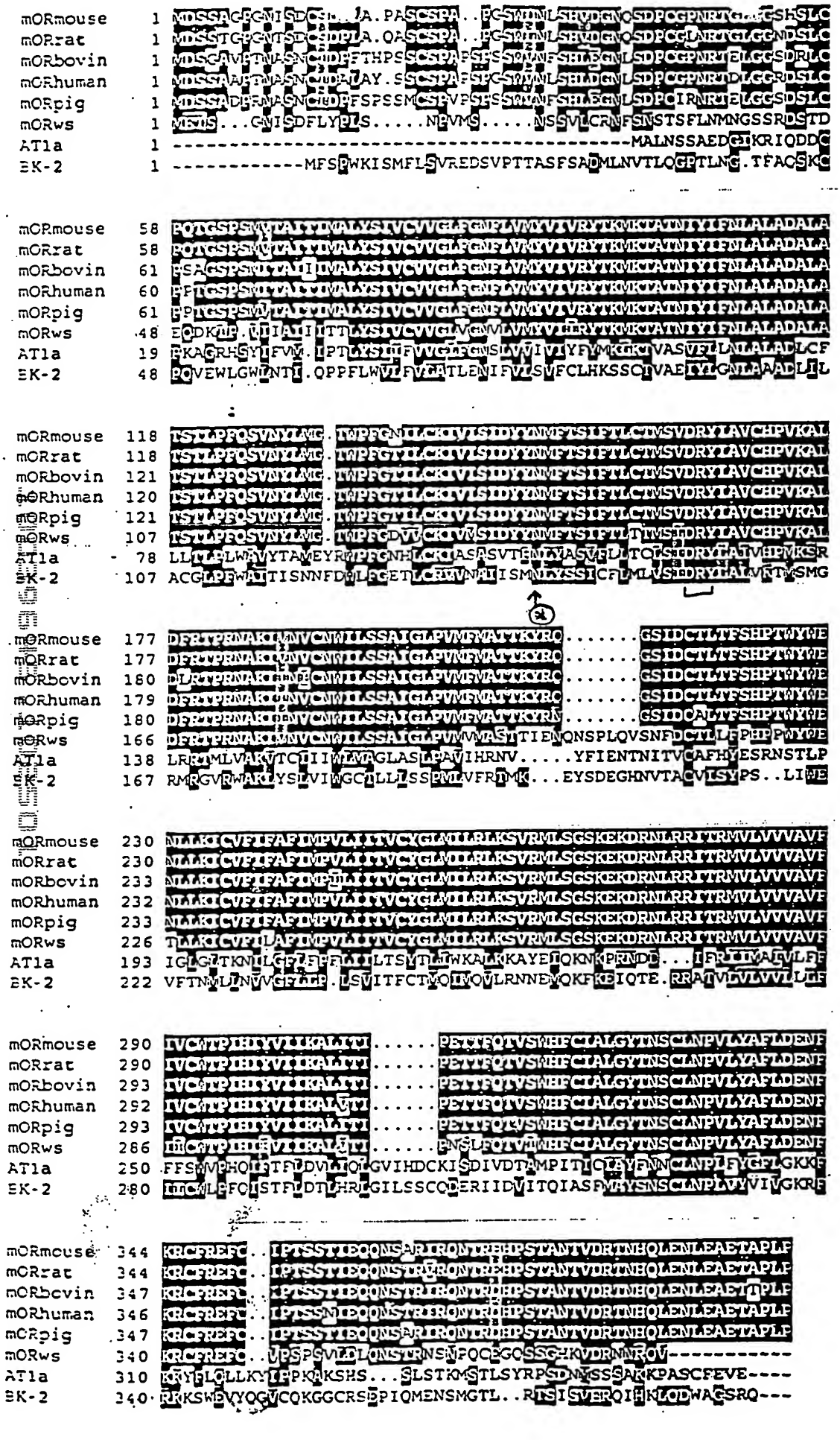


Figure 13


mORmouse
mORrat
mORbovin
mORhuman
mORpig
mORws
AT1a
EK-2

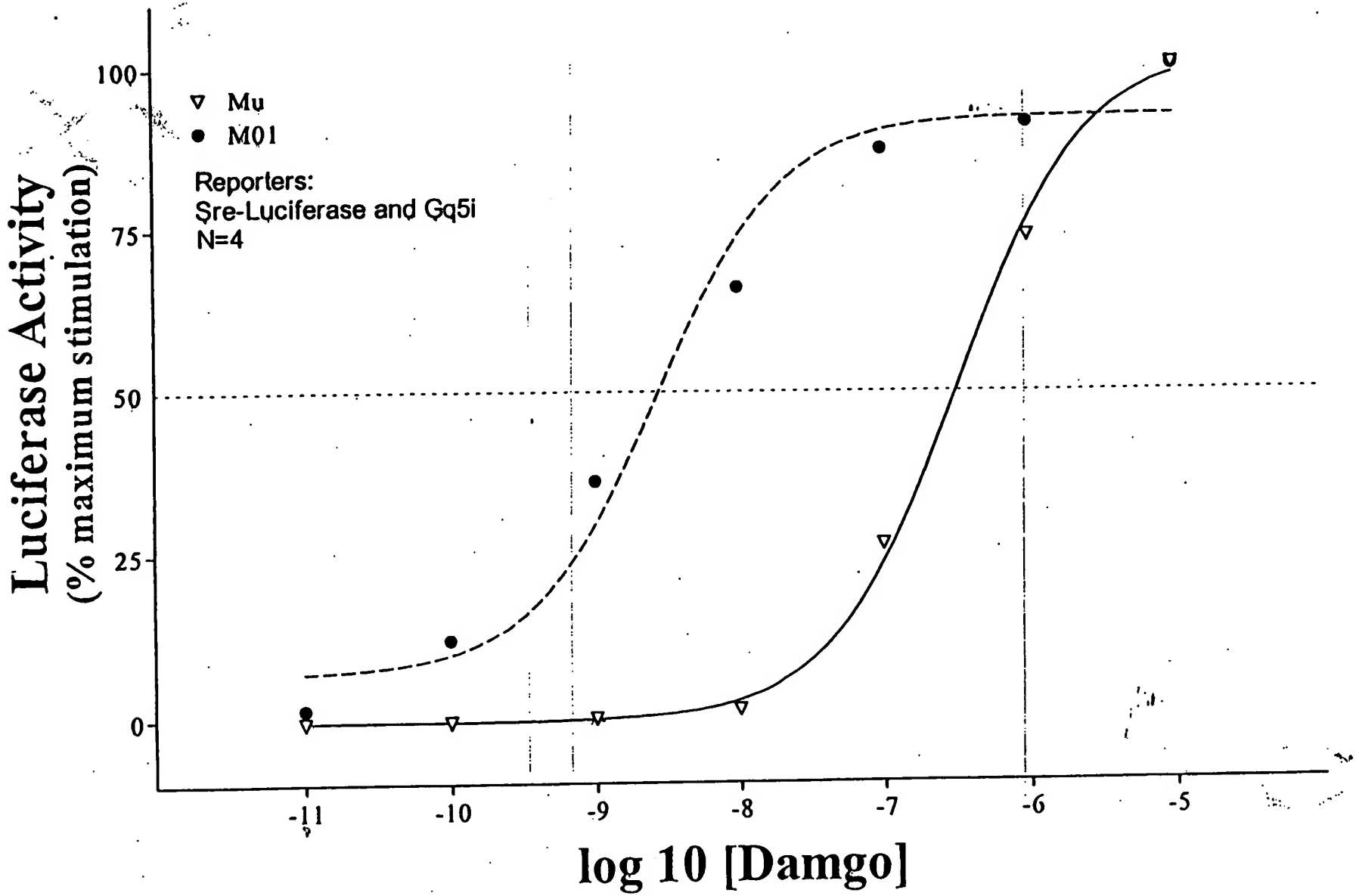

Mu
M01
Reporters:
Sre-Luciferase and Gq5i
N=4
Luciferase Activity
(% maximum stimulation)
100
75
50
25
0
-11
-10
-9
-8
-7
-6
-5
log 10 [Damgo]

Figure 15

An Intracellular Point Mutation Results in Loss of Ligand-Induced Function

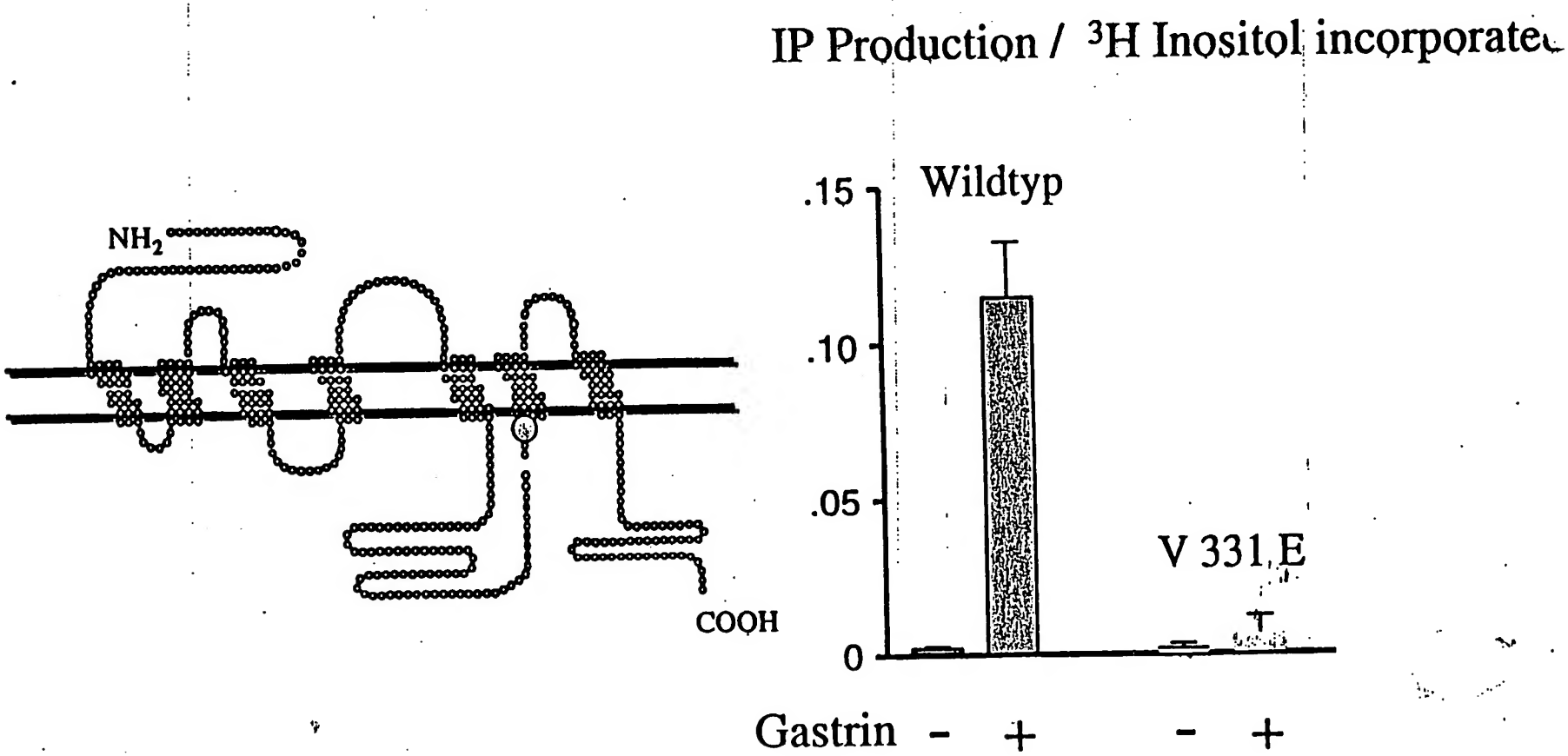


Figure 16

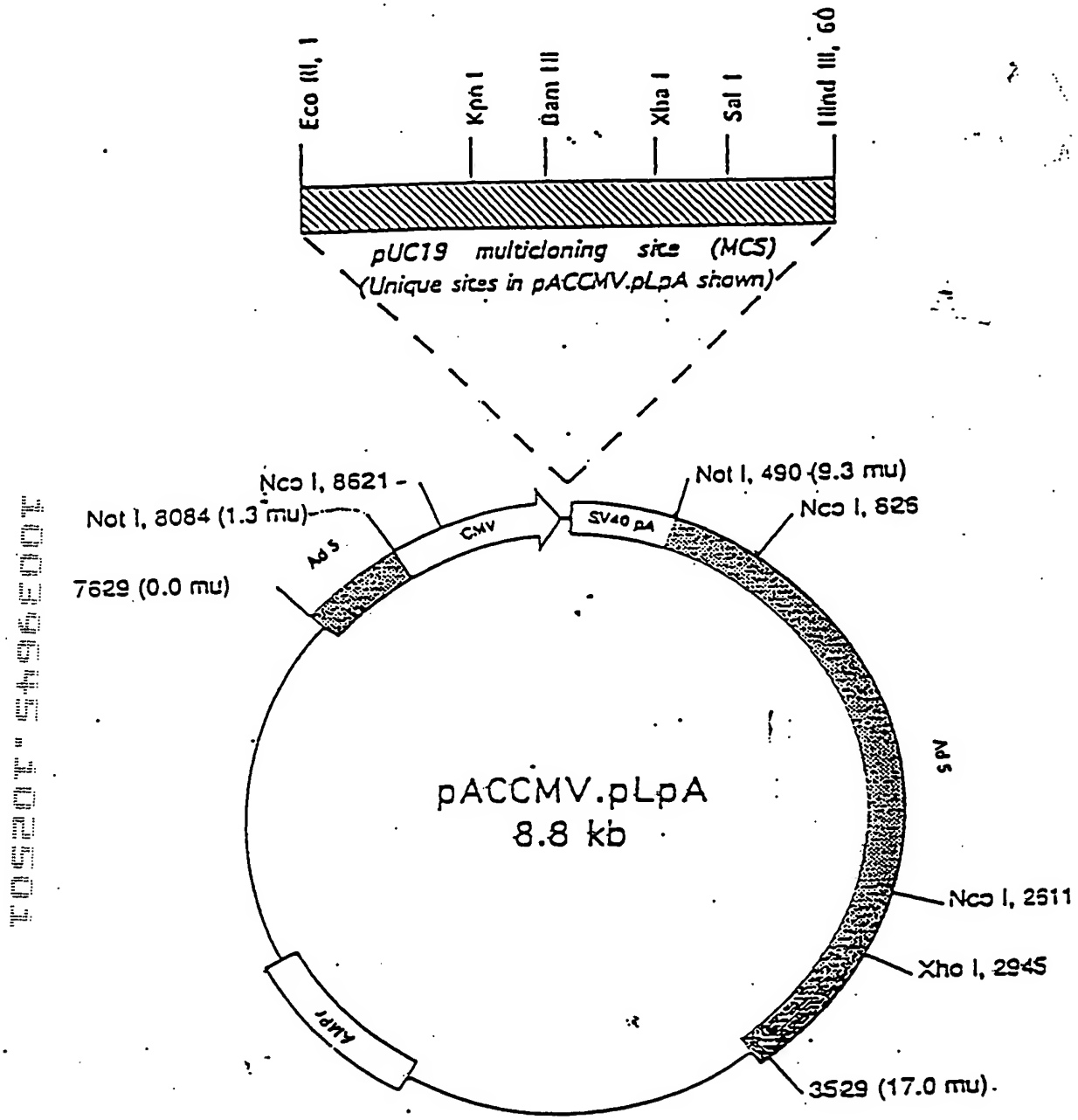

Eco III, 1
Kpn I
Bam III
Xba I
Sal I
IIInd III, 60
pUC19 multicloning site (MCS)
(Unique sites in pACCMV.pLpA shown)
Nco I, 8621
Not I, 8084 (1.3 mu)
7629 (0.0 mu)
CMV
SV40 pA
Not I, 490 (9.3 mu)
Nco I, 826
pACCMV.pLpA
8.8 kb
Nco I, 2511
Xho I, 2945
3529 (17.0 mu)
AMPr

Figure 17